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Purification of polysaccharide from *Solanum nigrum* L. by S-8 macroporous resin adsorption

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Abstract

To study the purification conditions of polysaccharide from *Solanum nigrum* L. by S-8 macroporous adsorption resin. The effect of multi-factors on the purification of polysaccharide from Solanum nigrum were studied by static - dynamic adsorption and desorption methods with indexes of adsorption and desorption rates. The optimized purification process condition was as follows: the concentration of sample was 5 mg/mL, pH level was 7.0, temperature was 30 °C, the adsorption velocity was 0.5 mL/min, the maximun loading amount was 10 BV, 0.5 mol/L NaCl solution 5BV was used as eluent, elution flow rate was 4 mL/min. With this condition, the purity of Solanum nigrum polysaccharide was 91.00%, which was 1.989 times higher than before. S-8 macroporous resin can be used to separate and purify the solanum nigrum polysaccharide and improve the purity and quality of polysaccharides.

Keywords: solanum nigrum polysaccharide; macroporous resin; purification; polysaccharide; resin adsorption

Practical Application: Purification of polysaccharide from Solanum nigrum L. by S-8 macroporous resin adsorption.

1 Introduction

As a dry herb of *Solanum nigrum* L., solanum nigrum belongs to solanaceae genus and nightshade, with properties of poisonous, cold and bitter, little sweet and effect of heat-clearing away and detoxifying, promoting circulation and removing stasis and reducing water swelling. Main active ingredient of solanum nigrum are alkaloids, polysaccharides and saponins. Recent studies reported that solanum nigrum has the effect of antimicrobial activity (Abbas et al., 2014), anti-tumor (Nawab et al., 2012; Ding et al., 2012; Wang et al., 2011; An et al., 2006) and immunomodulatory (Razali et al., 2016; Rahimi et al., 2010). However, less studies on the purification of solanum nigrum were reported.

Macroporous resin is a new organic polymer adsorbent with macroporous structure but without exchange group, with preferential adsorption of organic material by physical adsorption, which has been widely used in biological medicine, food, industrial and other fields (Wang & Wang, 2006; Li & Chase, 2010). For further development and application of solanum nigrum polysaccharide, S-8 macroporous resin was used on solanum nigrum polysaccharide by static-dynamic adsorption and desorption experiment based on the previous studies of screening experiment of macroporous resin and provide scientific basis for the extraction and purification technology of solanum nigrum polysaccharide.

2 Materials and instruments

Solanum nigrum medical slices, bought from Zhejiang Schwab Pharmaceutical co., LTD., was identified in accordance with Chinese pharmacopoeia (catalog number 20150720). Anhydrous glucose standard was from Chinese Medicine Shanghai Chemical Reagent Company. S-8, D3520, HPD-450 macroporous adsorption resin were from Cangzhou Baoen chemical co., LTD. Phenol, sodium hydroxide, hydrochloric acid, sulfuric acid, ethyl ether, anhydrous ethanol and other conventional reagents, are the domestic analytical pure. BS224S type electronic analytical balance was bought from Beijing Sartorius. JHBE-50T type extractors was from Henan Jinding technology). RV10-V type rotary evaporation instrument was from IKA Germany. SHZ-B type water-bathing constant temperature vibrator was from Shanghai Boxun Co. LTD. I3 type UV-VIS spectrophotometer was from Jinan Hanon Instrument Co. LTD.

2.1 Experimental methods

Preparation of solanum nigrum polysaccharide solution

Solanum nigrum medicine was smashed and sieved, adding 4 times the amount of petroleum ether, degreasing 2 h with 50 °C reflux for 2 times. Dried the residue, then placed in the flash type extractors, adding 30 times the amount of water, flash extraction with 80V extract voltage for 2 mins for 2 times. Merged the extracted liquid, filtration, concentrated to 1/2 amount of medicinal herbs. Then took supernatant after centrifugation, adding ethanol to the alcohol content of 80%. After refrigerated standing at 4 °C for one night, vacuum suction filter, precipitation with ethanol, ether in turn twice, vacuum drying at 60 °C, then the solanum nigrum polysaccharide was prepared. Took precise amount of solanum nigrum polysaccharide, adding water and dissolving under the condition of ultrasonic, a

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certain concentration solanum nigrum polysaccharide solution was prepared for further use.

Establishment of standard curve of solanum nigrum polysaccharide

The preparation of standard solution: take a certain amount of standard anhydrous glucose after drying at 105 °C. Placed the solution in a 500 mL volumetric flask after dissolving with water. Thin up to scale and shake well to get the contrast glucose solution with the concentration of 0.1 mg/mL.

The preparation of standard curve: precisely took 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.7, 0.8 mL of the contrast glucose solution in 10 mL volumetric flask respectively. Adding water to 2.0 mL and then adding 1.0 mL of 6% phenol solution. After shaking up, added 5.0 mL of sulfuric acid. Heated in boiling water bath for 15 mins and cool to room temperature. Use water as blank control group. Measured the absorbance at 490nm with ultraviolet spectrophotometer. Draw the regression curve by taking glucose concentration C as the abscissa and absorbance A as the ordinate. Calculate the regression equation as A = 17.42 + 0.019 C (R2 = 0.993). Results showed that the concentration of glucose with a good liner relationship in the range of 0.005 ~ 0.05 mg/mL.

The pre-treatment and packing of macroporous adsorption resin

Embathing the macroporous adsorption resin with distilled water for 2-3 times to eliminate the small and broken resin powder. Macroporous adsorption resin was wet packing after soaking in 95% ethanol for 24 h. Elute with ethanol till the elution didn't have white pollution after mixed with equal volume of water. And there is no obvious absorption peak in 200-400 nm range scanning ultraviolet spectrum. Then elute with 5% HCl solution, wash with distilled water till neutral. Elute with 5% NaOH solution and wash with distilled water till neutral. Set aside for further use.

Static adsorption and desorption experiment of S-8 resin

Static adsorption kinetics test

2.00 g S-8, D3520, HPD-450 macroporous adsorption resin was precise weighed and put in the conical flask respectively. And then add 60 mL of known concentration solanum nigrum polysaccharide in the conical flask. Put it in the 30 °C constant temperature water bath oscillator for 10 h with 120 r/min. Take 2 mL supernatant every 30 min and supplement 2 mL water to keep the same total volume. After that, absorbance was measured at 490 nm by using a microplate reader (Biotek, USA). The content and of adsorption polysaccharide were calculated. Make the plot of static adsorption kinetics with time as the abscissa and adsorption rate as the ordinate.

Effect of samples' mass concentration on static adsorption

10 copies of conical flask with 1.00 g (wet weight) S-8 macroporous adsorption resin were prepared. And then added 10 mL solanum nigrum polysaccharide with 0.25, 0.5, 1, 2, 2.5, 5, 10, 15, 20, 25 mg/mL the mass concentration respectively. Put them in the 30 °C constant temperature water bath oscillator for 10 h with 120 r/min. After suction filtration, the absorbance of supernatant was measured. The content and adsorption polysaccharide were calculated.

Effects of liquid-solid ratio on static adsorption

7 copies of conical flask with 1.00 g (wet weight) S-8 macroporous adsorption resin were prepared. And then added 5 mg/mL mass concentration of solanum nigrum polysaccharide with 10, 15, 20, 25, 30, 35, 40 mL respectively. Put them in the 30 °C constant temperature water bath oscillator for 10 h with 120 r/min. After suction filtration, the absorbance of supernatant was measured. The content and adsorption polysaccharide were calculated.

Effects of PH value on static adsorption

7 copies of conical flask with 1.00 g (wet weight) S-8 macroporous adsorption resin were prepared. And then added 5 mg/mL mass concentration of solanum nigrum polysaccharide with PH 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 respectively. Put them in the 30 °C constant temperature water bath oscillator for 10 h with 120 r/min. After suction filtration, the absorbance of supernatant was measured. The content and adsorption polysaccharide were calculated.

Effects of temperature on static adsorption

5 copies of conical flask with 1.00 g (wet weight) S-8 macroporous adsorption resin which was blotted up the surface water were prepared. And then each added 5 mg/mL mass concentration of solanum nigrum polysaccharide. Put them in the 25 °C, 30 °C, 35 °C, 40 °C, 45 °C constant temperature water bath oscillator respectively for 10 h with 120 r/min. After suction filtration, the absorbance of supernatant was measured. The content and adsorption polysaccharide were calculated.

Effect of eluent type on desorption effect

Put 1.00 g S-8 macroporous adsorption resin which was completed the static adsorption test in the conical flask. Dried surface of resin with filter paper. And added 30 mL water, 30 mL ethyl-alcohol and 30 mL NaCl solution respectively. The concentration of ethyl-alcohol was 20%, 30%, 40%, 50%, 60%, 70%, 80% and 90%. And the concentration of NaCl solution was 0.5 mol/L, 1 mol/L, 2 mol/L, 3 mol/L respectively. Put them in the 30 °C constant temperature water bath oscillator for 10 h with 120 r/min. After suction filtration, the absorbance of supernatant was measured. The content and adsorption polysaccharide were calculated.

Calculation of adsorption rate and desorption rate

Solanum nigrum polysaccharide sample liquid was diluted properly. According to the method of determination of absorbance under 2.2, and regression equation to calculate the content of polysaccharide. Calculated absorption and desorption rate on the basis of following Equations 1 to 4.

$$Q(mg/g) = (C0 - C1) \times V1/m \quad (1)$$

$$E(\%) = (C0 - C1)/C0 \times 100\% \quad (2)$$

$$Z(mg/g) = C2 \times V2/m \quad (3)$$

$$D(\%) = Z \times 100\%/Q \quad (4)$$

Q indicates the amount of adsorption, i.e., the amount of solanum nigrum polysaccharide which was absorbed by 1.00 g macroporous adsorption resin. E indicates adsorption rate, i.e., the adsorption percentage of macroporous adsorption resin (%). Z indicates desorption content, content, i.e., the amount of solanum nigrum polysaccharide which was desorbed by 1.00 g macroporous adsorption resin. D indicates desorption rate, i.e., the desorption percentage of S-8 macroporous adsorption resin (%). C0 indicates the mass concentration of polysaccharide solution before adsorption. C1 indicates the mass concentration of polysaccharide solution after adsorption. V1 indicates the volume of polysaccharide solution. M indicates the weight of macroporous adsorption resin. C2 indicates mass concentration of polysaccharide solution in the eluent after desorption. V2 indicates the volume of eluent.

The dynamic adsorption and desorption test of S-8 macroporous adsorption resin

Effect of velocity on dynamic adsorption

20 g of S-8 resin was wet packed. The same amount of solanum nigrum polysaccharide samples were with 0.5, 1, 2, 3, 4 mL/min flow rate for dynamic adsorption. The column effluent was collected and the absorbance of supernatant was measured. The content and adsorption polysaccharide were calculated.

Evaluation of maximum adsorption

20 g of S-8 resin was wet packed and 500 mL solanum nigrum polysaccharide samples (1 mg/mL) into the pillar with 0.5 mL/min flow rate for dynamic adsorption. Column effluents were collected for each BV fraction and total 14 samples. Then the absorbance of supernatant was measured. The content of polysaccharide were calculated. Dynamic adsorption curve were draw by taking the volume of samples as the abscissa, polysaccharide concentration as the ordinate

Elution velocity effect on resin and desorption

20 g of S-8 resin was wet packed. The same amount of solanum nigrum polysaccharide samples were with same flow rate for dynamic adsorption. Then elute macroporous adsorption resin with 0.5 mol/L NaCl with 0.5, 1, 2, 3, 4 mL/min flow rate. The eluent was collected and the absorbance of supernatant was measured. The content and desorption rate polysaccharide were calculated.

Effect of elute dosage on resin desorption

20 g of S-8 resin was wet packed. Take 350 mL of solanum nigrum polysaccharide samples with a certain flow rate for dynamic adsorption. The column effluent was collected and the absorbance of supernatant was measured. The content of polysaccharide was calculated. Then elute macroporous adsorption resin with 0.5 mol/L NaCl with a certain flow rate. One eluent was collected for each bed volume (BV), 18 samples collected in total. The absorbance of supernatant was measured. The content and desorption rate polysaccharide were calculated. With the dosage of eluent as the abscissa and desorption rate as the ordinate, dynamic elution curve was drawing.

Determination of solanum nigrum polysaccharide purity after adsorption purified by S-8 resin

S-8 macroporous adsorption resin was wet packed. Apply adsorption purification process on solanum nigrum polysaccharide with the best condition of adsorption and desorption from the above operation. The eluent was collected. The content of polysaccharide and the eluent volume was determined. Extract the eluent with rotary evaporation and freeze drying to constant weight. Calculated the content of purified polysaccharide and the enrichment factor of the purified solanum nigrum polysaccharide on the basis of following Equations 5 to 7. Repeat parallel test for 3 times.

$$X_1(\%) = (V_1 \times C_0) \times 100\%/m_1 \quad (5)$$

$$X_2(\%) = (V_2 \times C_2) \times 100\%/m_2 \quad (6)$$

$$Y(\%) = X_2 \times 100\%/X1 \quad (7)$$

X_1 indicates the purity of crude polysaccharide before purification (%); X_2 indicates the purity of pure polysaccharide before purification (%); C_0 indicates mass concentration polysaccharide solution before purification (mg/mL); V_1 indicates sample volume (mL); C_2 indicates the mass concentration of polysaccharide of eluent (mg/mL); V_2 indicates the eluent volume (mL); m1 indicates the mass of crude polysaccharide; m_2 indicates the mass of pure polysaccharide.

3 Results

3.1 Static adsorption experiment

Static adsorption kinetic curve

Results showed in Figure 1 after studied the adsorption effect of these 3 resins comprehensively that all these 3 resins were quick balance type for the adsorption of solanum nigrum polysaccharide. And it was helpful for industrial production. On the early stage of adsorption, the adsorption rate increased rapid with time increased and the resin almost reached to saturated adsorption after 2.5 h and became gentle. The S-8 macroporous

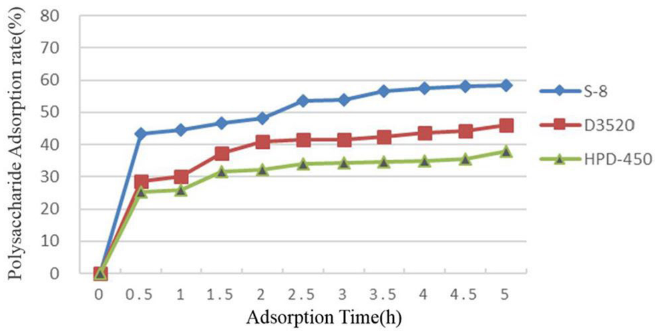

Figure 1. Static kinetic curves of 3 macroporous resin.

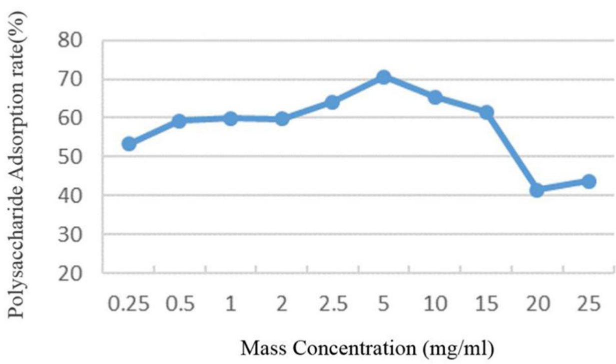

Figure 2. The effect of polysaccharide concentration on S-8 absorption rate.

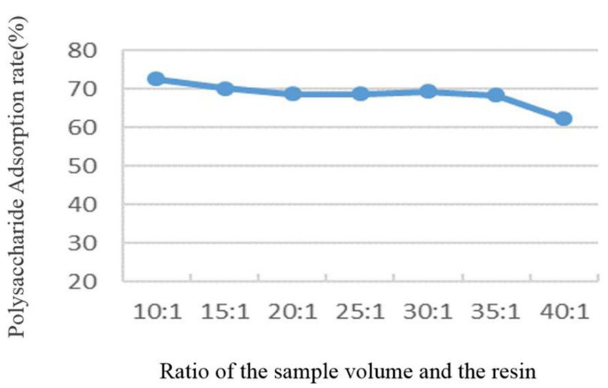

Figure 3. The effect of liquid to solid ratio on S-8 absorption rate.

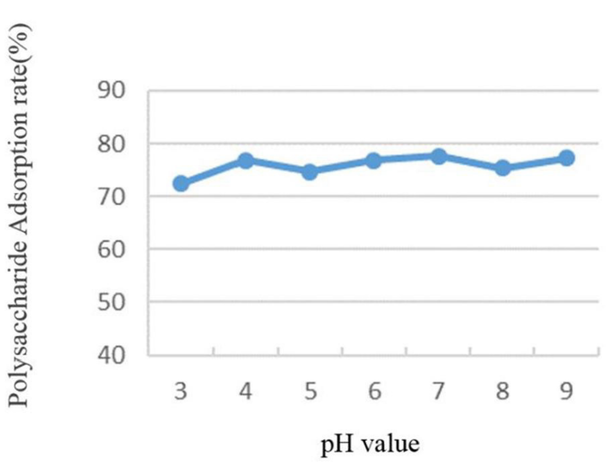

Figure 4. Effect of pH value on S-8 absorption rate.

resin was chosen for purifition process study since the adsorption rate of this resin was highest.

The effect of mass concentration of sample solution on dynamic adsorption results

Results showed that different mass concentration of sample solution had a big influence on adsorption effect of macroporous resin. In general, a lower mass concentration was much helpful for the adsorption capacity of macroporous resin. As showed in Figure 2, the adsorption rate of macroporous resin increased with sample mass concentration increased when mass concentration was low. The resin and adsorption reached adsorption equilibrium when the mass concentration of solanum nigrum polysaccharide was 5.0 mg/mL, where the adsorption rate of polysaccharide was the largest. The adsorption capacity decreased with the mass concentration increased continuously. When considering the efficiency of the resin, a proper mass concentration of samples was set as 5.0 mg/mL.

The effect of liquid-solid ratio on dynamic adsorption results

As showed in Figure 3, when the dosage of resin was certain, polysaccharide adsorption rate was highest when the ratio of the sample volume and the resin is 10:1. The adsorption rate decreased when sample volume was too much. The reason was that excessive sample volume was over the saturated adsorption of resin resulted in the loss of part of the polysaccharide. So the most appropriate liquid-solid ratio was 10:1, about 6 bed volume.

The effect of pH value on the kinetic adsorption results

In general, pH value is an important factor for adsorption. As showed in Figure 4, polysaccharide adsorption rate was highest in neutral environment when pH was 7.

The effect of temperature on kinetic adsorption

As results showed in Figure 5. For a certain adsorption system, it couldn't reach to a balance in a short time at low temperature. It was helpful to improve the adsorption with increasing temperature. Due to adsorption was exothermic reaction, desorption rate increased when temperature was too high and was unhelpful for adsorption. So the adsorption temperature need to be appropriate. The adsorption rate of S-8 resin on solanum nigrum polysaccharide increased firstly, then gradually decreased. The adsorption rate was highest at 30 °C (Figure 5).

The effect of eluent type on desorption results

As showed in Figure 6, the eluent type and concentration would have an important influence on desorption of polysaccharide. When using ethanol as eluent, desorption increased when ethanol volume fraction increased. When using NaCl as eluent, desorption decreased with the concentration increased. The desorption rate reached at maximum, up to 67.89%, when the NaCl mass concentration was 0.5 mol/L.

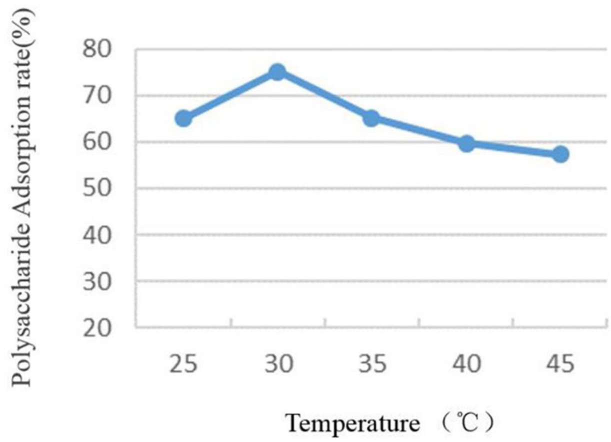

Figure 5. Effect of temperature on S-8 absorption rate.

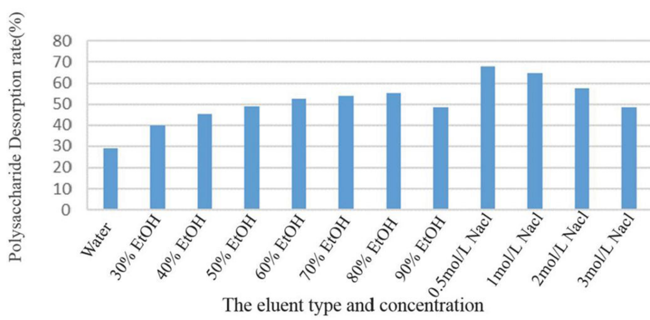

Figure 6. Effect of eluant type on S-8 desorption rate.

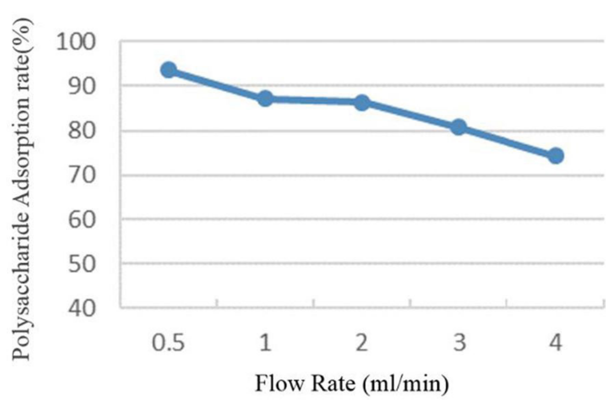

Figure 7. The effect of sample flow rate on S-8 absorption rate.

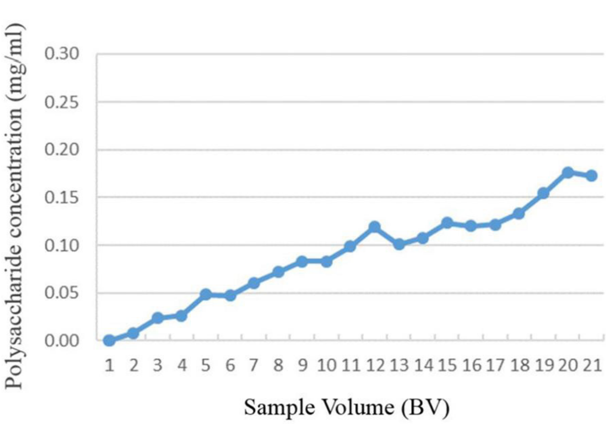

Figure 8. Dynamic adsorption curve.

3.2 The kinetic adsorption and desorption experiments of S-8 resin

The effect of flow rate on kinetic adsorption results

As showed in Figure 7, when the flow rate was too fast, the polysaccharide molecules couldn't fully spread to the surface of the resin due to the insufficient explosion between resin and polysaccharide molecules, thus resulted in adsorption decreased with flow rate increased. When the flow rate was too slow, the adsorption rate was affected due to the long adsorption time. Result showed in Figure 7 that the adsorption rate of polysaccharide decreased gradually with an increasing velocity. Considering the maximum adsorption of solanum nigrum polysaccharide, the best velocity was 0.5 mL/min.

The kinetic adsorption curve

As showed in Figure 8, when the sample volume was low, the concentration of eluate polysaccharide was low and increased gradually with the sample volume increased. The concentration of eluate increased rapidly when the sample volume was over 10BV. The adsorption of S-8 resin on polysaccharide was tend to be saturated. So the adsorption of resin reached to the maximum as 3.46 mg/g when sample volume was 10BV.

The effect of eluate flow rate on kinetic desorption results

As result showed in Figure 9. The eluate flow rate had an important effect on desorption of resin. The elution strength was insufficient and polysaccharide couldn't completely detached from the resin adsorption if the flow rate was too slow. The insufficient explosion between eluate and polysaccharide and was unable to adsorpt polysaccharide if the flow rate was too fast.

The kinetic elution curve

The desorption of polysaccharide need a process. The desorption rate increased with the eluate dosage increased when the eluate volume was low. As showed in Figure 10, the desorption rate reached to maximum when the eluate dosage was 2BV. The desorption rate decreased gradually with increasing eluate dosage and the desorption process was basically completed that the polysaccharide concentration of eluate was almost 0 when was 5BV. So the best eluate volume was 5BV.

3.3 The effect of S-8 macroporous resin on the purification of solanum nigrum polysaccharide.

The confirmatory test results showed that the purity of these three solanum nigrum polysaccharide were improved from 45.75% to 91.29%, 91.44% and 90.26% after purified by S-8 macroporous resin. The average purity was 91.00% and polysaccharide concentration reached 1.989 times. The results indicated that the effective purification process was stable and with good reproducibility and suitable for the adsorption and purification of solanum nigrum polysaccharide.

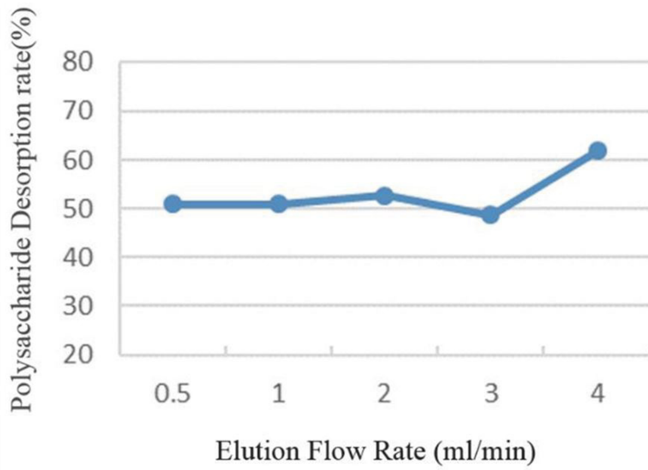

Figure 9. The effect of elution flow rate on S-8 desorption.

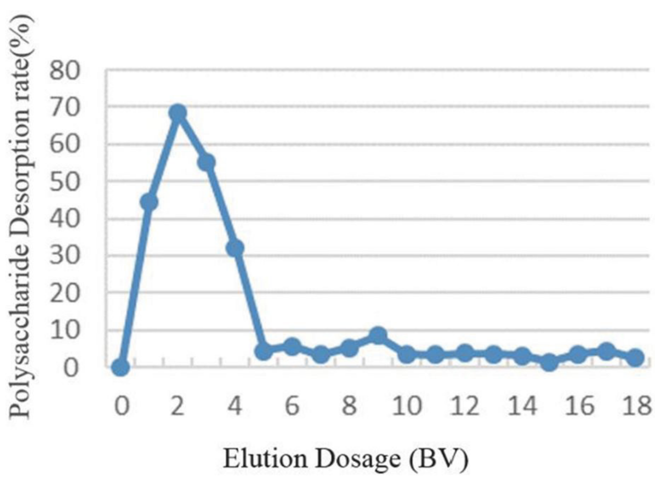

Figure 10. Dynamic elution curve.

4 Discussion

Macroporous adsorption resins have been widely used in the separation and purification of targeted component or in the removal of the impurities from the samples (Hatano et al., 2009; Liu et al., 2011). The adsorption mechanism of S-8 macroporous adsorption resin is that it's a hyper-cross-linked polar resin with extremely rigid networks and based on dipole-dipole forces because of the polar derivation compounds (Żwir-Ferenc & Biziuk, 2006). Purification of the polysaccharide is based on the precipitation of protein with chloroform and *n*-butanol, while it more weakly decolored the sample compared to S-8 resin (Jung et al., 2001).

Generally the purity of polysaccharide was difficult and the purification method was complicated (Yang et al., 2012). Macroporous adsorption resin has higher adsorption capacity, fast adsorption, mild desorption conditions, easy regeneration process, long use cycle, cost saving, and many other advantages, and was widely used in separation and purification of Chinese herbal medicine component (Zou et al., 2015; Dong et al., 2015; Yang et al., 2016). The purity of macroporous adsorption resin on solanum nigrum polysaccharide was studied in this research. The optimal purification conditions were determined through static-dynamic test: the mass concentration of samples was 5 mg/mL, pH value was 7.0, temperature was 30 °C, the sample volume was 10BV, sample flow rate was 0.5 mL/min, eluting with 0.5 mol/L NaCl, eluate flow rate was 4 mL/min and eluate volume was 5BV. The results showed that the polysaccharide concentration was significant improved and the purity reached 91.00% and polysaccharide concentration reached 1.989 times after the S-8 macroporous resin adsorption process.

This study provided the raw material for structure determination and structure-activity relationship study of solanum nigrum polysaccharide and had a great significance for the utilization and the development of new drugs of solanum nigrum polysaccharide. This achievement may also be helpful for further structural and pharmacological research of the purified polysaccharide from many pharmaceutical plant.

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